UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

MEMORANDUM

Date: August 27, 2015

Subject: Efficacy Review for Sting
EPA Reg. Number: 777-114
DP Barcode: 427483

From: Thao Pham
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Product Science Branch
Antimicrobials Division (7510P)

To: Eric Miederhoff, Team 31
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Reckitt Benkiser LLC
Scientific & Regulatory Consultants
P.O. Box 1014
Columbia City, IN 46725

Formulation from the Label:

Active Ingredient(s)	% by wt.
Alkyl (C_{12} 40%; C_{14} 50%; C_{16}10%) dimethyl benzyl ammonium chloride	0.260%
Other Ingredients	99.740%
Total	100.000%

I BACKGROUND

The product, Sting (EPA Reg. No. 777-114), is an EPA-approved disinfectant (bactericide, virucide), sanitizer, fungistat, and deodorizer for use on hard, non-porous surfaces in household, commercial, and institutional environments. The applicant is submitting efficacy data to support claims against additional organisms on the label. Label directions indicate that the product is effective as a "one-step" disinfectant. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121 (recently renamed as Accuratus Lab Services).

This data package contains a letter from the registrant, Reckitt Benckiser LLC, to EPA (dated May 8, 2015), a proposed label, and fourteen studies (MRID 49622301-49622314). A Statement of No Data Confidentiality Claims, Good Laboratory Practice Statement, and Quality Assurance Unit Summary were included in with each study.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: appliance exteriors, baby carriages, baseboards, bed frames, booster seats, cabinets, computer, countertop, cribs, diaper changing tables, diaper pails, doorknobs, E-readers/electronics, exhaust fans, fitness equipment, floors, gaming consoles, garbage cans, highchair, kitchen surfaces, oven, outdoor furniture, rail, remote control, shelves, showers, sinks, sports equipment, stovetops, table tops, telephones, toilets, toys, tubs, and urinals. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: enamel tile, finished wood, glazed ceramic tile, glazed porcelain tile, hard plastic, and stainless steel. Directions on the proposed label provide the following information regarding preparation and use of the product as a sanitizer/disinfectant:

To Sanitize / Disinfect: Pre-clean surface. Use enough fresh (wipes) (wet) (cloths) to thoroughly wet surface. Surface must remain wet for the entire contact time. **To Sanitize:** Allow to remain wet for (10) (or) (30) seconds. **To Disinfect:** Allow to remain wet for (4) (or) (10) minutes. Allow surface to air dry. Toss dirty wipe away. (Rinse all food contact surfaces and toys with water after use.)

For surfaces that come in contact with food: Use only on hard, non-porous surfaces and rinse thoroughly with water.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

<u>Antimicrobial Products for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes (Additional Bacteria):</u>

The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test. Ten carriers should be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the glass slide, the product should be tested by wiping the surface of the glass slide with the saturated towelette, and then subculturing the slides after the specified holding time. The towelette should be removed from its container and subsequently handled with sterile

gloves. One towelette should be used to wipe a minimum of 10 inoculated slides. One carrier with a surface area equivalent to ten 1 x 1 inch carriers or ten carriers each with a surface area of 1 x 1 inch should be wiped using one towelette per carrier set (for a total of six towelettes and 60 carriers) per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test. The product should kill all the test microorganisms on all carriers/slides within ten minutes or less.

Supplemental Claims

An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

Virucides:

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. If the product is intended to be represented as a one-step virucidal, an appropriate organic soil (i.e.- 5 percent blood serum) should be included with the viral inoculum.

Sanitizers (For Non-Food Contact Surfaces; Additional Bacteria):

There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is to be used as a sanitizer for non-food contact surfaces. Confirmatory test standards would apply. Therefore, 2 product samples, representing 2 different product lots, should be tested against each additional microorganism. The ASTM method states that the inoculum employed should provide a count sufficient to demonstrate at least 99.9 percent over the parallel control within 5 minutes.

IV SYNOPSIS OF SUBMITTED EFFICACY STUDY

1. MRID 49622301 "Standard Test Method for Efficacy of Sanitizers: Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-saturated Towelette Product Application, Test Organism: *Campylobacter jejuni* (ATCC 29428)" for Sting, by Joshua Leudtke, M.S. Study conducted at ATS Labs. Study completion date – January 30, 2015. Project Identification Number: A17662.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Two lots (Lot Nos. 1934-155 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC81110614.NFS.1 dated November 6, 2014 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 3 day old suspension of test organism (targeted to 1 x 10^8 CFU/mL). Test culture was thoroughly mixed prior to testing. The culture was spread evenly to within 3 mm of the edges of the carrier and allowed to dry in a petri dish with the lids slightly ajar for 20 minutes at 27.5°C and 65% relative humidity. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions. After drying, each carrier was wiped with the saturated towelette back and forth twice, for a total of four passes. The area of the towelettes used was rotated so as to expose a maximum amount of towelette surface during the course of the wiping procedure. One towelette was used to treat all 5 carriers. The carriers were allowed to remain wet for 10 seconds at 21°C and 14% relative humidity. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 to neutralize and vortex mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto the recovery agar plate medium. All subcultures were incubated for 4 days at 35-37°C, under microaerophilic conditions. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

2. MRID 49622302 "Standard Test Method for Efficacy of Sanitizers: Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-saturated Towelette Product Application, Test Organism: *Listeria monocytogenes* (ATCC 19111)" for Sting, by Joshua Leudtke, M.S. Study conducted at ATS Labs. Study completion date – January 30, 2015. Project Identification Number: A17663.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 1934-155 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC81110614.NFS.2 dated November 6, 2014 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 48-54 hour old suspension of test organism. Test culture was thoroughly mixed prior to testing. The culture was spread evenly to within 3 mm of the edges of the carrier and allowed to dry in a petri dish with the lids slightly ajar for 20 minutes at 27.5°C and 47% relative humidity. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions. After drying, each carrier was wiped with the saturated towelette back

and forth twice, for a total of four passes. The area of the towelettes used was rotated so as to expose a maximum about of towelette surface during the course of the wiping procedure. One towelette was used to treat all 5 carriers. The carriers were allowed to remain wet for 10 seconds at 21°C and 14% relative humidity. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 to neutralize and vortex mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto the recovery agar plate medium. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

3. **MRID 49622303 "Standard Test Method for Efficacy of Sanitizers: Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-saturated Towelette Product Application, Test Organism: Hospital Acquired Methicillin Resistant *Staphylococcus aureus (*HA-MRSA) (ATCC 33591)" for Sting, by Kristen Niehaus, B.A. Study conducted at ATS Labs. Study completion date – February 6, 2015. Project Identification Number: A17671.**

This study was conducted against Hospital Acquired Methicillin Resistant *Staphylococcus aureus (*HA-MRSA) (ATCC 33591). Two lots (Lot Nos. 1934-155 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC81110614.NFS.5 dated November 6, 2014 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 48-54 hour old suspension of test organism. Test culture was vortex mixed and allowed to stand for ≥ 15 minutes prior to testing. Upper 2/3rds of the culture was removed and mixed thoroughly prior to use. The culture was spread evenly to within 3 mm of the edges of the carrier and allowed to dry in a petri dish with the lids slightly ajar for 21 minutes at 36.2°C and 40% relative humidity. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions. After drying, each carrier was wiped with the saturated towelette back and forth twice, for a total of four passes. The area of the towelettes used was rotated so as to expose a maximum about of towelette surface during the course of the wiping procedure. One towelette was used to treat all 5 carriers. The carriers were allowed to remain wet for 10 seconds at 21°C and 29% relative humidity. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 to neutralize and vortex mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto the recovery agar plate medium. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Hospital Acquired Methicillin Resistant *Staphylococcus aureus (*HA-MRSA) (ATCC 33591) was verified on a representative culture. Two 1 µg oxacillin antibiotic disks were placed flat directly onto the surface of a Mueller Hinton agar plate inoculated with HA-MRSA (ATCC 33591). Within 15 minutes of application, the plates were inverted and incubated at 35-37°C for ≥24 hours. Following incubation, the zone of inhibition showing no visible growth was measured. If no zone was present, the size of the disc was reported. The examination confirmed

antibiotic resistance according to the Clinical and Laboratory Standards Institute. See pages 9 and 19 of the laboratory report.

4. **MRID 49622304 "Standard Test Method for Efficacy of Sanitizers: Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-saturated Towelette Product Application, Test Organism: Hospital Acquired Methicillin Resistant *Staphylococcus aureus (*HA-MRSA) (ATCC 33592)" for Sting, by Kristen Niehaus, B.A. Study conducted at ATS Labs. Study completion date – February 6, 2015. Project Identification Number: A17670.**

This study was conducted against Hospital Acquired Methicillin Resistant *Staphylococcus aureus (*HA-MRSA) (ATCC 33592). Two lots (Lot Nos. 1934-155 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC81110614.NFS.4 dated November 6, 2014 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 48-54 hour old suspension of test organism. Test culture was vortex mixed and allowed to stand for ≥ 15 minutes prior to testing. Upper 2/3rds of the culture was removed and mixed thoroughly prior to use. The culture was spread evenly to within 3 mm of the edges of the carrier and allowed to dry in a petri dish with the lids slightly ajar for 20 minutes at 36.8°C and 42% relative humidity. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions. After drying, each carrier was wiped with the saturated towelette back and forth twice, for a total of four passes. The area of the towelettes used was rotated so as to expose a maximum about of towelette surface during the course of the wiping procedure. One towelette was used to treat all 5 carriers. The carriers were allowed to remain wet for 10 seconds at 21°C and 29% relative humidity. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 to neutralize and vortex mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto the recovery agar plate medium. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Hospital Acquired Methicillin Resistant *Staphylococcus aureus (*HA-MRSA) (ATCC 33591) was verified on a representative culture. Two 1 µg oxacillin antibiotic disks were placed flat directly onto the surface of a Mueller Hinton agar plate inoculated with HA-MRSA (ATCC 33591). Within 15 minutes of application, the plates were inverted and incubated at 35-37°C for ≥24 hours. Following incubation, the zone of inhibition showing no visible growth was measured. If no zone was present, the size of the disc was reported. The examination confirmed antibiotic resistance according to the Clinical and Laboratory Standards Institute. See pages 9 and 19 of the laboratory report.

5. **MRID 49622305 "Standard Test Method for Efficacy of Sanitizers: Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-saturated Towelette Product Application, Test Organism: *Salmonella enterica* (ATCC 10708)" for Sting, by Kristen Niehaus, B.A. Study conducted at ATS Labs. Study completion date – February 9, 2015. Project Identification Number: A17669.**

This study was conducted against *Salmonella enterica* (ATCC 10708). Two lots (Lot Nos. 1934-155 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC81110614.NFS.3 dated November 6, 2014 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 48-54 hour old suspension of test organism. Test culture was thoroughly mixed prior to testing. The culture was spread evenly to within 3 mm of the edges of the carrier and allowed to dry in a petri dish with the lids slightly ajar for 21 minutes at 36.1°C and 40% relative humidity. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions. After drying, each carrier was wiped with the saturated towelette back and forth twice, for a total of four passes. The area of the towelettes used was rotated so as to expose a maximum about of towelette surface during the course of the wiping procedure. One towelette was used to treat all 5 carriers. The carriers were allowed to remain wet for 10 seconds at 21°C and 25% relative humidity. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 to neutralize and vortex mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto the recovery agar plate medium. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

6. **MRID 49622306 "Standard Test Method for Efficacy of Sanitizers: Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-saturated Towelette Product Application, Test Organism: *Streptococcus pyogenes* (ATCC 19615)" for Sting, by Joshua Leudtke, M.S. Study conducted at ATS Labs. Study completion date – January 30, 2015. Project Identification Number: A17661.**

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 1934-155 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC81110614.NFS.6 dated November 6, 2014 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) glass slide carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 3 day old suspension of test organism. Test culture was thoroughly mixed prior to testing. The culture was spread evenly to within 3 mm of the edges of the carrier and allowed to dry in a petri dish with the lids slightly ajar for 20 minutes at 36.2°C and 40% relative humidity. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions. After drying, each carrier was wiped with the saturated towelette back and forth twice, for a total of four passes. The area of the towelettes used was rotated so as to expose a maximum about of towelette surface during the course of the wiping procedure. One towelette was used to treat all 5 carriers. The carriers were allowed to remain wet for 10 seconds at 21°C and 43%

relative humidity. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 to neutralize and vortex mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution were plated onto the recovery agar plate medium. All subcultures were incubated for 48±4 hours at 35-37°C in 6% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

7. **MRID 49622307 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: Rotavirus (ATCC VR-2018, Strain WA)" for Sting, by Ritu Upadhyaya, M.T. Study conducted at Accuratus Lab Services. Study completion date – March 11, 2015. Project Identification Number: A17919.**

This study was conducted against Rotavirus (ATCC VR-2018, Strain WA) using Rhesus monkey kidney (RMK) cells (MA-104) (obtained from the American Type Culture Collection (ATCC CRL-2378.1)) as the host system. Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula # e0002-078, were tested using ATS Labs protocol # SRC81020915.ROT dated February 9, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Three (3) sterile glass petri dish carriers (150 x 15 mm) per product lot were inoculated with 200 µL of test organism. The culture was spread evenly over an approximate 8 cm x 8 cm area on the bottoms of the petri dish and dried for 20 minutes at 20°C and 40% relative humidity, until visibly dry. For each lot of test substance, using sterile gloves, each carrier was divided in two sections and held covered at 20.0°C for the 10 minute exposure time. Each carrier was treated by wiping the virus film with the towelette over and back two times for a total of four passes. Each section was wiped so overlapping was minimal. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface in the course of wiping each section of the carrier. Following the exposure time, a 2.00 mL aliquot of test medium was added to each petri dish, and the dishes were scraped with a plastic cell scraper to re-suspend the contents (10-' dilution). The mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers. The filtrates (10^{-1} dilution) were immediately titered by 10-fold serial dilution and each dilution was then assayed for infectivity and/or cytotoxicity. The RMK cell line which exhibits CPE in the presence of Rotavirus, was used as the indicator cell line in the infectivity assays. Cells contained in multiwell cell culture were inoculated in quadruplicate with 100 µL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the absence or presence of cytopathic effect, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

8. **MRID 49622308 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: Avian Influenza A (H3N2) Reassortant Virus (ATCC VR-2072, Strain A/Washington/897/80 x A/Mallard/New York/6750/78)" for Sting, by Sharon Conway, B.S. Study conducted at Accuratus Lab Services. Study completion date – April 27, 2015. Project Identification Number: A18195.**

This study was conducted against Avian Influenza A (H3N2) Reassortant Virus (ATCC VR-2072, Strain A/Washington/897/80 x A/Mallard/New York/6750/78), using MDCK (canine

kidney) cells (obtained from the American Type Culture Collection, (ATCC CCL-34)) as the host system. Two lots (Lot Nos. 1934-161 and 1934-167) of the product Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.AFLU dated March 16, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the virus stock culture to achieve a 5% organic load. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over a defined area of approximately 8 x 8 cm on the bottom of three separate 150 x 15 mm sterile glass petri dishes. The virus films were air dried for 20 minutes at 20.0°C and 40.0% relative humidity, until visibly dry. Using sterile gloves, each towelette was used to wipe each carrier back and forth twice, for a total of four passes. The carriers were allowed to remain wet while covered for 2 minutes at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, utilizing syringe plungers in order to detoxify the mixtures. The filtrations were then diluted serially and assayed for infectivity and/or cytotoxicity. The MDCK cell line which exhibits CPE in the presence of Avian Influenza A (H3N2) Reassortant virus, was used as the indicator cell line in the infectivity assays. Cells contained in multiwell cell culture were inoculated in quadruplicate with 100 µL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the absence or presence of cytopathic effect, cytotoxicity, and viability. All cultures were microscopically examined and results were recorded. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

9. MRID 49622309 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Streptococcus mutans* (ATCC 125175)" for Sting, by Ritu Upadhyaya, M.T. Study conducted at Accuratus Lab Services. Study completion date – April 28, 2015. Project Identification Number: A18196.

This study was conducted against *Streptococcus mutans* (ATCC 125175). Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.TOW.2 dated March 16, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inches x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Test culture was vortex mixed for 3-4 seconds and allowed to stand for ≥ 10 minutes prior to testing. Upper portion of the culture was removed and mixed thoroughly prior to use. The culture was spread evenly over an approximate 1 inch x 1 inch area on the end of the slide contained in the Petri dish. The dish was immediately covered and the carriers were dried for 30 minutes at 27°C and 65% relative humidity, until visibly dry. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier was wiped back and forth twice, for a total of four passes. The carriers were allowed to remain wet for 4 minutes at 21°C and 27% relative humidity. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time. Following the exposure period, excess liquid was drained off the carrier and individual carriers were transferred to 20 mL of Brain Heart Infusion Broth +0.07% Lecithin +0.5% Tween 80 to neutralize. Each vessel was shaken thoroughly. All subcultures were incubated for 2-4 days at 35-37°C in the presence of CO_2. Following incubation,

the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

10. MRID 49622310 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Escherichia coli* O157:H7 (ATCC 51657)" for Sting, by Jamie Herzan, B.S. Study conducted at Accuratus Lab Services. Study completion date – April 27, 2015. Project Identification Number: A18183.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 43888). Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.TOW.11 dated April 27, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inches x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Test culture was vortex mixed for 3-4 seconds and allowed to stand for ≥ 10 minutes prior to testing. Upper portion of the culture was removed and mixed thoroughly prior to use. The culture was spread evenly over an approximate 1 inch x 1 inch area on the end of the slide contained in the Petri dish. The dish was immediately covered and the carriers were dried for 32 minutes at 36.1°C and 55.4% relative humidity, until visibly dry. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier was wiped back and forth twice, for a total of four passes. The carriers were allowed to remain wet for 4 minutes at 20.44°C and 26.87% relative humidity. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time. Following the exposure period, excess liquid was drained off the carrier and individual carriers were transferred to 20 mL of Letheen Broth + 0.07% Lecithin +0.5% Tween 80 to neutralize. Each vessel was shaken thoroughly. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

11. MRID 49622311 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Streptococcus algalactiae* (ATCC 13813)" for Sting, by Jamie Herzan, B.S. Study conducted at Accuratus Lab Services. Study completion date – April 27, 2015. Project Identification Number: A18178.

This study was conducted against *Streptococcus algalactiae* (ATCC 13813). Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.TOW.3 dated March 16, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inches x 1 inch) per product lot were inoculated with 10.0 µL of a 48-54 hour old suspension of test organism. Test culture was vortex mixed for 3-4 seconds and allowed to stand for ≥ 10 minutes prior to testing. Upper portion of the culture was removed and mixed thoroughly prior to use. The culture was spread evenly over an approximate 1 inch x 1 inch area on the end of the slide contained in the Petri dish. The dish was immediately covered and the carriers were dried for 30 minutes at 27°C and 65% relative humidity, until visibly dry. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated

so as to expose a maximum amount of its surface during wiping. Each carrier was wiped back and forth twice, for a total of four passes. The carriers were allowed to remain wet for 4 minutes at 21°C and 30% relative humidity. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time. Following the exposure period, excess liquid was drained off the carrier and individual carriers were transferred to 20 mL of Letheen Broth + 0.07% Lecithin +0.5% Tween 80 to neutralize. Each vessel was shaken thoroughly. All subcultures were incubated for 2 days at 35-37°C in the presence of 6.0% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

12. MRID 49622312 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Salmonella enterica* subspecies *enterica* serovar Gallinarum (ATCC 9184)" for Sting, by Jamie Herzan, B.S. Study conducted at Accuratus Lab Services. Study completion date – April 27, 2015. Project Identification Number: A18197.

This study was conducted against *Salmonella enterica* subspecies *enterica* serovar Gallinarum (ATCC 9184). Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.TOW.5 dated March 16, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inches x 1 inch) per product lot were inoculated with 10.0 µL of a 2 day old suspension of test organism. Test culture was suspended in Butterfield's buffer to approximately match a 1.0 McFarland turbidity standard. The final culture was mixed thoroughly prior to use. The culture was spread evenly over an approximate 1 inch x 1 inch area on the end of the slide contained in the Petri dish. The dish was immediately covered and the carriers were dried for 32 minutes at 26.9-27.0°C and 65% relative humidity, until visibly dry. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier was wiped back and forth twice, for a total of four passes. The carriers were allowed to remain wet for 4 minutes at 21°C and 30% relative humidity. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time. Following the exposure period, excess liquid was drained off the carrier and individual carriers were transferred to 20 mL of Letheen Broth + 0.07% Lecithin +0.5% Tween 80 to neutralize. Each vessel was shaken thoroughly. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

13. MRID 49622313 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Staphylococcus hominis* (ATCC 25615)" for Sting, by Ritu Upadhyaya, M.T. Study conducted at Accuratus Lab Services. Study completion date – April 28, 2015. Project Identification Number: A18181.

This study was conducted against *Staphylococcus hominis* (ATCC 25615). Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.TOW.8 dated March 16, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a

pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inches x 1 inch) per product lot were inoculated with 10.0 µL of a 2-3 day old suspension of test organism. A test organism suspension of 4.0 McFarland was prepared using Butterfield's Buffer and mixed thoroughly prior to use. The culture was spread evenly over an approximate 1 inch x 1 inch area on the end of the slide contained in the Petri dish. The dish was immediately covered and the carriers were dried for 30 minutes at 36.0-36.2°C and 53.8% relative humidity, until visibly dry. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier was wiped back and forth twice, for a total of four passes. The carriers were allowed to remain wet for 4 minutes at 21°C and 27% relative humidity. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time. Following the exposure period, excess liquid was drained off the carrier and individual carriers were transferred to 20 mL of Letheen Broth + 0.07% Lecithin +0.5% Tween 80 to neutralize. Each vessel was shaken thoroughly. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Due to faint growth in neutralization and viability control, all subcultures were further re-incubated for 2 days at 35-37°C. Following re-incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

14. MRID 49622309 "Pre-Saturated Towelettes for Hard Surface Disinfection, Test Organism: *Streptococcus suis* (ATCC 43765)" for Sting, by Ritu Upadhyaya, M.T. Study conducted at Accuratus Lab Services. Study completion date – May 1, 2015. Project Identification Number: A18177.

This study was conducted against *Streptococcus suis* (ATCC 43765). Two lots (Lot Nos. 1934-161 and 1934-167) of the product, Sting, EPA Reg. No. 777-114, Formula# e0002-078, were tested using ATS Labs protocol # SRC91031615.TOW.1 dated March 16, 2015 (copy provided). The tested active ingredient concentration reported in the study is acceptable in accordance with lower certified limit on the CSF. The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers (3 inches x 1 inch) per product lot were inoculated with 10.0 µL of a 2-3 day old suspension of test organism. Test culture was suspended in Fluid Thioglycollate Medium to target $1x10^8$ CFU/mL and mixed thoroughly prior to use. The culture was spread evenly over an approximate 1 inch x 1 inch area on the end of the slide contained in the Petri dish. The dish was immediately covered and the carriers were dried for 30 minutes at 27°C and 65% relative humidity, until visibly dry. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier was wiped back and forth twice, for a total of four passes. The carriers were allowed to remain wet for 4 minutes at 21°C and 31% relative humidity. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time. Following the exposure period, excess liquid was drained off the carrier and individual carriers were transferred to 20 mL of Brain Heart Infusion Broth +0.07% Lecithin +0.5% Tween 80 to neutralize. Each vessel was shaken thoroughly. All subcultures were incubated for 2-4 days at 35-37°C in the presence of CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

V RESULTS

STING (EPA Reg. No. 777-114)
Sanitization – Bactericidal Efficacy – 10 second contact time

MRID No.	Organism	Lot No.	Total Quat (%)	Carrier Population (Average Log_{10}/ Carrier)	Test Results (Average Log_{10}/Carrier)	Percent Reduction
49622301	*Campylobacter jejuni* (ATCC 29428)	1934-155	0.214	4.81	< 1.30	> 99.9
		1934-167	0.216		< 1.30	> 99.9
49622302	*Listeria monocytogenes* (ATCC 19111)	1934-155	0.214	7.27	< 1.30	> 99.9
		1934-167	0.216		< 1.30	> 99.9
49622303	Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (ATCC 33591)	1934-155	0.214	6.80	< 1.30	> 99.9
		1934-167	0.216		< 1.30	> 99.9
49622304	Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)	1934-155	0.214	6.85	< 1.30	> 99.9
		1934-167	0.216		< 1.30	> 99.9
49622305	*Salmonella enterica* (ATCC 10708)	1934-155	0.214	5.09	< 1.30	> 99.9
		1934-167	0.216		< 1.30	> 99.9
49622306	*Streptococcus pyogenes* (ATCC 19615)	1934-155	0.214	5.96	< 1.30	> 99.9
		1934-167	0.216		< 1.30	> 99.9

Disinfection – Virucidal Efficacy

MRID Number	Organism	Contact Time	Results			Average Dried Virus Count
				Lot No. 1934-161	Lot No. 1934-167	
49622307	Rotavirus (ATCC VR-2018)	10 minutes	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/100µL
			$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
49622308	Avian Influenza A H3N2 (ATCC VR-2072)	2 minutes	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/100µL
			$TCID_{50}$/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	

Disinfection – Bactericidal Efficacy – 4-Minute Contact Time

MRID Number	Organism	Lot No.	Total Quat (%)	No. Exhibiting Growth/Total No. Tested	Average Log_{10} (CFU/ carrier)
49622309	*Streptococcus mutans* (ATTC 25175)	1934-161	0.195	0/10	4.53
		1934-167	0.205	0/10	
49622310	*Escherichia coli* O157:H7 (ATCC 51657)	1934-161	0.212	0/10	5.47
		1934-167	0.216	0/10	
49622311	*Streptococcus agalactiae* (ATCC 13813)	1934-161	0.212	0/10	4.83
		1934-167	0.216	0/10	
49622312	*Salmonella enterica* subspecies *enterica* serovar Gallinarum (ATCC 9184)	1934-161	0.212	0/10	5.37
		1934-167	0.216	0/10	
49622313	*Staphylococcus hominis* (ATCC 25615)	1934-161	0.195	0/10	5.86
		1934-167	0.205	0/10	
49622314	*Streptococcus suis* (ATCC 43765)	1934-161	0.195	0/10	5.46
		1934-167	0.205	0/10	

VI CONCLUSIONS

1. The submitted efficacy data **supports** the use of the product, Sting, as a sanitizer with bactericidal activity against the following microorganisms on hard, inanimate, non-porous surfaces for a 10-second contact time:

   Campylobacter jejuni (ATCC 29428) MRID: 49622301
   Listeria monocytogenes (ATCC 19111) MRID: 49622302
   Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (ATCC 33591) MRID: 49622303
   Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) MRID: 49622304
   Salmonella enterica (ATCC 10708) MRID: 49622305
   Streptococcus pyogenes (ATCC 19615) MRID: 49622306

   According to the Certificates of Analysis, the concentrations of the active ingredient in all test lots were at the lower certified limit. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms.

2. The submitted efficacy data **supports** the use of the product, Sting, as a disinfectant with virucidal activity against the following microorganisms on hard, inanimate, non-porous surfaces for a 10-minute contact time:

   Rotavirus (ATCC VR-2018) MRID: 49622307

   According to the Certificates of Analysis, the concentrations of the active ingredient in all test lots were at the lower certified limit. Acceptable log reduction was observed in all subcultures tested against the required number of product lots.

3. The submitted efficacy data **supports** the use of the product, Sting, as a disinfectant with virucidal activity against the following microorganisms on hard, inanimate, non-porous surfaces for a 2-minute contact time:

   Avian Influenza A H3N2 (ATCC VR-2072) MRID: 49622308

   According to the Certificates of Analysis, the concentrations of the active ingredient in all test lots were at the lower certified limit. Acceptable log reduction was observed in all subcultures tested against the required number of product lots.

4. The submitted efficacy data **supports** the use of the product, Sting, as a disinfectant with bactericidal activity against the following microorganisms on hard, inanimate, non-porous surfaces for a 4-minute contact time:

Streptococcus mutans (ATTC 25175)	MRID 49622309
Escherichia coli O157:H7 (ATCC 51657)	MRID 49622310
Streptococcus agalactiae (ATCC 13813)	MRID 49622311
Salmonella enterica subspecies enterica serovar Gallinarum (ATCC 9184)	MRID 49622312
Staphylococcus hominis (ATCC 25615)	MRID 49622313
Streptococcus suis (ATCC 43765)	MRID 49622314

   According to the Certificates of Analysis, the concentrations of the active ingredient in all test lots were at the lower certified limit. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms.

VII LABEL

1. The proposed label claims that the product, Sting, is an effective sanitizer with bactericidal activity against the following on hard, non-porous surfaces for a 10-second contact time:

   Campylobacter jejuni (ATCC 29428)
   Listeria monocytogenes (ATCC 19111)
   Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (ATCC 33591)
   Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)
   Salmonella enterica (ATCC 10708)
   Streptococcus pyogenes (ATCC 19615)

   These claims are **acceptable** as it is supported by the submitted data.

2. The proposed label claims that the product, Sting, is an effective disinfectant with virucidal activity against the following on hard, non-porous surfaces for a 10 minute contact time:

   Rotavirus (ATCC VR-2018)

   These claims are **acceptable** as they are supported by the submitted data.

3. The proposed label claims that the product, Sting, is an effective disinfectant with virucidal activity against the following on hard, non-porous surfaces for a 2 minute contact time:

   Avian Influenza A H3N2 (ATCC VR-2072)

   These claims are **acceptable** as they are supported by the submitted data.

4. The proposed label claims that the product, Sting, is an effective disinfectant with bactericidal activity against the following on hard, non-porous surfaces for a 4 minute contact time:

   Streptococcus mutans (ATTC 25175)
   Escherichia coli O157:H7 (ATCC 51657)
   Streptococcus agalactiae (ATCC 13813)
   Salmonella enterica subspecies enterica serovar Gallinarum (ATCC 9184)
   Staphylococcus hominis (ATCC 25615)
   Streptococcus suis (ATCC 43765)

   These claims are **acceptable** as they are supported by the submitted data.

5. On page 12 of the label, remove the term “household” for household bacteria.

6. Remove “protect” and “protection” claims from the label (see page 13 of label).